



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07C 403/24, C12P 23/00		A1	(11) International Publication Number: WO 98/03480
			(43) International Publication Date: 29 January 1998 (29.01.98)
(21) International Application Number: PCT/EP97/03961 (22) International Filing Date: 18 July 1997 (18.07.97)		(74) Agents: VISSER-LUIJINK, Gesina et al.; Gist-Brocades N.V., Patents and Trademarks Dept., Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft (NL).	
(30) Priority Data: 96202035.0 19 July 1996 (19.07.96) EP (34) Countries for which the regional or international application was filed: NL et al. 96202334.7 22 August 1996 (22.08.96) EP (34) Countries for which the regional or international application was filed: NL et al. 96202852.8 15 October 1996 (15.10.96) EP (34) Countries for which the regional or international application was filed: NL et al.		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): GIST- BROCADE B.V. [NL/NL]; Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft (NL).		Published <i>With international search report.</i>	
(72) Inventors; and (75) Inventors/Applicants (for US only): SIBEYN, Mieke [NL/NL]; Pauwstraat 8, NL-3816 AV Amersfoort (NL). DE PATER, Robertus, Mattheus [NL/NL]; Verkadestraat 6, NL-2613 ZL Delft (NL).			
<p>(54) Title: PROCESS FOR THE RECOVERY OF CRYSTALLINE β-CAROTENE FROM A NATURAL SOURCE</p> <p>(57) Abstract</p> <p>The present invention describes a process to recover highly pure β-carotene crystals from a crude crystal preparation obtained from a natural source. To remove impurities, the crude crystals are stirred in a solvent in which β-carotene has a low solubility, whereupon the crystals are filtered off and washed with fresh solvent. Using the process of the invention, a natural crystalline β-carotene preparation with a very high purity is obtained, a purity comparable to that of synthetic β-carotene.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Process for the recovery of crystalline β -carotene
from a natural source

5

Field of the invention

The present invention relates to the field of the recovery of carotenoids from a natural source.

10

Background of the invention

Currently, β -carotene crystals with a high purity (96% or higher) are produced by chemical synthesis. When derived from a natural source, β -carotene mostly is in the form of an oily extract (palm oil, algal oil). Although it is also possible to obtain β -carotene crystals from natural sources, such as vegetables (for example carrots) or micro-organisms (for example algae (*Dunaliella*) or fungi (*Blakeslea*)), the currently available processes to obtain relatively pure crystals from said natural sources have important disadvantages.

20 Current processes for the purification of β -carotene crystals from a natural source typically comprise an extraction step and additional purification steps.

25 The extraction is carried out with various extractants: organic solvents, such as ethyl acetate, butyl acetate or hexane, vegetable oils, or supercritical fluids, such as propane, ethylene, CO_2 .

To obtain a relatively pure β -carotene preparation, a further purification of the extract is necessary. Several purification processes have been described, among which chromatography, adsorption/desorption processes and crystallization or precipitation.

30 The β -carotene crystals which are directly crystallized from the extract

obtained after solvent extraction of a suitable natural source, e.g. by evaporation of the solvent, typically do not have the desired high purity, e.g. a purity compared to that of synthetic β -carotene. In such cases recrystallizations are required (NL 6411184, US 4,439,629). The main drawback of 5 recrystallization is that a large amount of solvent is required to solubilize the β -carotene. In addition, to recrystallize the β -carotene with a sufficiently high yield, large amounts of antisolvent (precipitating solvent) are necessary as well. Thus, these processes have the disadvantage that large amounts of solvents are required and a considerable loss of β -carotene can easily occur.

10

Summary of the invention

The present invention discloses a process for the preparation of β -carotene crystals with a purity of at least 90% from a natural source. Said 15 process comprises the following steps:

- * solvent-extraction of β -carotene from said natural source to produce a β -carotene extract,
- * crystallization of β -carotene from said extract to produce crude β -carotene crystals,
- 20 * treatment of said crude β -carotene crystals with a solvent selected from the group of solvents in which β -carotene has a low solubility,
- * optionally repeating said solvent treatment with the same or a different solvent in which β -carotene has a low solubility,
- 25 * evaporation of the residual solvent from the crystals.

Optionally, the β -carotene extract obtained after solvent extraction of a natural source is washed with water prior to crystallization of β -carotene.

In a preferred embodiment, the method of the invention provides a crystalline β -carotene preparation from a natural source with a purity of 30 $\geq 95\%$.

The method of the invention can be conveniently used to increase the β -carotene content of any impure crystalline β -carotene preparation.

Detailed description of the invention

The present invention discloses a process for the recovery of highly pure β -carotene crystals from a natural source with a high yield. According to the 5 invention, highly pure β -carotene crystals are recovered from crude β -carotene crystals using a simple and convenient solvent treatment. The process of the invention comprises a treatment of a crude β -carotene crystal preparation with a solvent in which β -carotene has a low solubility.

The crude β -carotene crystals which are subjected to the process of the 10 invention are obtained by conventional extraction and crystallization technology. Typically, the extraction of β -carotene from a natural source is carried out with organic solvents, vegetable oils or supercritical fluids. The subsequent crystallization or precipitation is performed by methods as cooling, evaporation of the solvent or addition of an antisolvent, followed by filtering off 15 the crystals.

The β -carotene content of the crude crystals mainly depends on the natural source which is used for isolation, whereas the applied crystallization procedure additionally can influence the same. Typically, said β -carotene content varies between 60 and 90%.

20 Surprisingly, the solvent treatment of the crude crystals according to the invention significantly increases the β -carotene content of the crystals. The crystals obtained after solvent treatment have a β -carotene content of $\geq 90\%$, preferably a β -carotene content of $\geq 92\%$, more preferably a β -carotene content of $\geq 94\%$.

25 In the process according to the invention, crude β -carotene crystals are treated with a solvent in which β -carotene has a low solubility. The solvent treatment of the invention comprises the steps of stirring the crude β -carotene crystals in said solvent during a time period sufficient to enable dissolution of impurities, filtering off the crystals and washing the crystals several times with 30 fresh solvent. Optionally, the treatment is repeated one or more times. After the final washing step, residual solvent is evaporated.

Solvents which are suitable to use in the treatment according to the

invention are solvents in which β -carotene has a low solubility, i.e. a solubility of at most 1 g/l at 25 °C. Preferably, the solvent is water or an organic solvent.

When water is used, the pH of the water is not a critical factor for the treatment of the invention, although it is preferred that the pH is below 7. More preferably, the pH of the water is 4-6.

The organic solvent preferably is a lower alcohol or a lower acyl ester thereof, wherein lower is understood to comprise a straight or branched chain of 1 to 5 carbon atoms, or acetone. More preferably, the organic solvent is ethanol or ethylacetate.

The solvent in which β -carotene has a low solubility is applied in a sufficient amount to remove a substantial amount of impurities. Preferably, the solvent to crystal ratio is \geq 5 ml solvent per gram crystal, more preferably \geq 5-10 ml per gram crystal, most preferably \geq 20-40 ml per gram crystal. It is understood that the upper limit of the volume of solvent per gram crystals is not a technical limit but is determined by economical criteria.

The time which is necessary for dissolution of the impurities in the solvent is dependent on the temperature at which the stirring is performed. Typically, the stirring is performed at a temperature below the boiling point of the solvent. Preferably, the stirring is performed at a temperature of 20 to 80 °C, more preferably 30 to 60 °C, most preferably 50 °C.

The solubility of β -carotene in the solvent which is used also may influence the temperature of the stirring process. When using a solvent in which β -carotene has a relatively low solubility, e.g. ethanol, the stirring preferably is performed at an elevated temperature, e.g. 50 °C, whereas when using a solvent in which β -carotene has a relatively high solubility, e.g. ethylacetate, the stirring temperature may be chosen in the lower range, e.g. 20 to 25 °C.

When using a solvent in which β -carotene has a relatively high solubility, it is a preferred option to keep the stirring temperature at a more elevated level, e.g. 50 °C, and to cool the mixture after stirring to a lower temperature, e.g. a temperature of 5 to 20 °C, to prevent relatively high losses of β -carotene.

At a temperature of 50 °C, a suitable stirring time is for instance about 30 minutes.

The present invention also envisages the option to subsequently treat crude β -carotene crystals with two or more different solvents in which β -carotene has a low solubility.

It is a further option to wash the extract obtained by solvent-extraction of a natural source with water prior to crystallization of β -carotene from said extract. In this embodiment, the amount of water used is not very critical, although it may be relevant to choose said amount such that a good phase separation is obtained.

The present invention provides a simple and convenient method to increase the β -carotene content of β -carotene crystals. The solvent treatment of the invention is advantageously applied to any crystalline β -carotene preparation obtainable from a natural source (plant or a microbial), of which the purity is not as high as desired. In addition, the method of the present invention is applicable to a crude crystal preparation of a natural carotenoid other than β -carotene.

Preferably, the method of the invention is applied to β -carotene crystals obtainable from a microbial source, more preferably from algae or fungi (including yeasts), even more preferably from fungi of the order *Mucorales*, most preferably from *Blakeslea trispora*.

In a preferred embodiment of the invention, highly pure, natural β -carotene crystals are obtainable from microbial biomass, e.g. from *Blakeslea trispora*. Said highly pure, natural, crystalline β -carotene preparation has a purity of $\geq 95\%$, preferably $\geq 96\%$, more preferably $\geq 97\%$, even more preferably $\geq 98\%$, most preferably $\geq 99\%$. Due to the natural origin of the highly pure, crystalline β -carotene preparation, said preparation may also contain a small amount of other carotenoids, such as γ -carotene. The content of γ -carotene thereby may vary from about 0.5 to about 1.5%.

A preferred process comprises the steps of washing an ethylacetate extract obtained from *Blakeslea trispora* with water prior to crystallization, crystallization of β -carotene from said washed extract to obtain crude crystals

- 6 -

and subsequent treatment of said crude crystals with two different solvents, i.e. firstly with ethylacetate and secondly with ethanol, both solvent treatments occurring at a temperature of 50 °C.

Thus, the method of the invention enables the manufacture of a
5 crystalline β -carotene preparation from a natural source which has an extremely high purity, i.e. a purity which is not obtained using conventional technology, i.e. a purity which is $\geq 95\%$.

The highly purified β -carotene crystals obtained by the process of the invention are suitable for food, pharmaceutical and cosmetic applications. The
10 natural β -carotene crystals typically are applied as a oily suspension, e.g. a 30% (w/v) suspension of crystals in a vegetable oil, such as soybean oil.

Example 1

Extraction of β -carotene from biomass with ethyl acetate

15

54 g of biomass of *Blakeslea trispora*, containing 4.3% of β -carotene was mixed with 600 ml of ethyl acetate (Merck, p.a.). The suspension was heated to 50°C and kept at that temperature during 2 hrs under stirring. Subsequently the suspension was filtrated, yielding an extract with a β -
20 carotene concentration of 1.7 g/l.

The β -carotene content of crystals is analyzed spectrophotometrically (FCC III/Monographs) or with proton-NMR.

Example 2

Isolation of highly pure β -carotene crystals from ethyl acetate extract

The extract obtained in Example 1 was concentrated to a β -carotene concentration of 12 g/l by evaporating the solvent at 50°C under vacuum. The
30 concentrate was cooled to 20 °C and kept at 20°C during 2 hours under stirring. The crystals were filtered and washed with two cake-volumes of ethyl acetate, yielding 0.7 gram of crude crystal with a purity of 91.9%.

- 7 -

Subsequently the dried crude crystals were stirred for 30 minutes in 35 ml of ethanol at 50°C. After cooling to room temperature, the crystals were filtered off, washed with 2 cake volumes of ethanol and dried under vacuum at room temperature. 0.65 gram of crystals with a purity of 97.0% were 5 obtained.

Example 3

Isolation of highly pure β -carotene crystals
from ethyl acetate extract

10

An extract containing 1.7 g/l of β -carotene was prepared as described in Example 1. This extract was concentrated to a β -carotene concentration of 10 g/l by evaporating the solvent at 50°C under vacuum. The concentrate was cooled to 5°C and kept at 5°C during two hours under stirring. The crystals 15 were filtered and washed with two cake-volumes of ethyl acetate, yielding 0.8 gram of crude crystal with a purity of 94.1%.

Subsequently the dried crude crystals were stirred for 30 minutes in 41 ml of ethanol at 50°C. After cooling to room temperature, the crystals were filtered off, washed with 2 cake volumes of ethanol and dried under vacuum at 20 room temperature. 0.78 gram of crystals with a purity of 97.3% were obtained.

Example 4

Extraction of β -carotene from biomass with hexane

25

60 g of biomass of *Blakeslea trispora*, containing 4.3% of β -carotene was mixed with 900 ml of hexane (Merck, p.a.). The suspension was heated to 50°C and kept at that temperature during 3 hrs under stirring. Subsequently the suspension was filtrated, yielding an extract with a β -carotene 30 concentration of 1.5 g/l.

Example 5Isolation of highly pure β -carotene from hexane extract

The extract obtained in Example 4 was concentrated to a β -carotene concentration of 10 g/l by evaporating the solvent at 50°C under vacuum. The concentrate was cooled to 5°C and kept at 5°C during 2 hours under stirring. The crystals were filtered and washed with two cake-volumes of hexane, yielding 0.94 gram of crude crystal with a purity of 87.9%.

Subsequently the dried crude crystals were stirred for 30 minutes in 35 ml of ethanol at 50°C. After cooling to room temperature, the crystals were filtered off, washed with 2 cake volumes of ethanol and dried under vacuum at room temperature. 0.85 gram of crystals with a purity of 98.0% were obtained.

15

Example 6Washing of crude β -carotene crystals with water

Crude crystals with a purity of 85.3% were obtained after extraction of dried Blakeslea trispora biomass with ethyl acetate at 50°C and a solvent to biomass ratio of 30/1, and subsequent concentration of the extract to a concentration of 15 g/l.

These crude crystals were suspended in demineralized water (20 g/l) and stirred for 30 minutes at 40°C. After filtering off the crystals, the crystals were washed with two cake volumes of ethanol at room temperature and dried under vacuum at room temperature.

Washing with demineralized water at pH 4 yields crystals with a purity of 96.2%, washing with demineralized water at pH 7 or pH 10 yields crystals with a purity of 92%.

30

Example 7Large scale isolation of highly pure β -carotene crystals

210 kg of dried biomass of Blakeslea trispora, containing 4.2% of β -carotene was mixed with 4200 l of ethyl acetate. The suspension was heated to 50°C and kept at that temperature during 3 hours under stirring. Subsequently the suspension was filtered, yielding an extract with a β -carotene concentration of 1.4 g/l.

The ethyl acetate extract was subsequently mixed with demineralized water of 50°C in a volumetric ratio of 10/1 (extract/water). After stirring during 15 minutes at 50°C, the layers were separated. The ethyl acetate layer was then concentrated to a β -carotene content of 6 g/l by evaporation.

The concentrate was cooled to 5°C, causing crystallization of β -carotene. After two hours at 5°C under stirring, the crystals were filtered and washed with two cake volumes of ethyl acetate, yielding 6.4 kg of wet crystals (dry matter 60.2%). The wet crystals were stirred in 75 l of ethanol at 50°C during 30 minutes. The suspension was then allowed to cool to 20°C, the crystals were filtered and subsequently washed with two cake volumes of ethanol. The crystals were finally dried under vacuum at room temperature, yielding 3.6 kg of crystals with a purity of 97.2%.

Example 8Large scale isolation of highly pure β -carotene crystals

25 400 kg of dried biomass of Blakeslea trispora, containing 4.2% of β -carotene was mixed with 12 m³ of ethyl acetate. The suspension was heated to 50°C and kept at that temperature during 8 hours under stirring. Subsequently the suspension was filtered, yielding an extract with a β -carotene concentration of 1.7 g/l.

30 The ethyl acetate extract was subsequently mixed with demineralized water of 50°C in a volumetric ratio of 5/1. After stirring during 15 minutes at 50°C, the layers were separated. The ethyl acetate layer was then

- 10 -

concentrated to a β -carotene content of 4 g/l by evaporation.

The concentrate was cooled to 5°C, causing crystallization of β -carotene. After two hours at 5°C under stirring, the crystals were filtered and washed with two cake volumes of ethyl acetate, yielding 24 kg of wet crystals
5 (dry matter 55%). The wet crystals were subsequently stirred in 450 l of ethyl acetate at 50°C during 30 minutes. The suspension was then allowed to cool to 5°C, the crystals were filtered and subsequently washed with two cake volumes of ethyl acetate and finally one cake volume of ethanol. 23 kg of wet crystals were obtained.

10 Next the crystals were stirred in 450 l of ethanol (95%) at 50°C during 30 minutes. After cooling of the suspension to 20°C, the crystals were filtered and washed with two cake volumes of ethanol, yielding 23 kg of wet crystals. The crystals were finally dried under vacuum at room temperature, yielding 13.8 kg of crystals with a purity of 100.4% (spectrophotometric method of
15 FCC III).

Claims

1. A process for the preparation of β -carotene crystals with a purity of \geq 90% from a natural source, comprising the steps of:

- 5 * solvent-extraction of β -carotene from said source to produce a β -carotene extract,
- * crystallization of β -carotene from said extract to produce crude β -carotene crystals,
- * treatment of said crude β -carotene crystals with a solvent selected from the group of solvents in which β -carotene has a low solubility,
- 10 * optionally repeating the previous solvent treatment with the same or a different solvent in which β -carotene has a low solubility,
- * evaporation of the residual solvent from the crystals.

15

2. The process of claim 1, wherein a first solvent treatment of crude β -carotene crystals is followed by a second treatment with a solvent different from the first solvent.

20

3. The process of claim 1 or 2, wherein the treatment of said crude β -carotene crystals comprises the steps of:

- * stirring said crude β -carotene crystals in said solvent,
- * filtering off the crystals,
- * washing the crystals with fresh solvent.

25

4. The process of any one of the claims 1-3, wherein the solvent in which β -carotene has a low solubility is water.

30

5. The process of any one of the claims 1-3, wherein the solvent in which β -carotene has a low solubility is an organic solvent.

6. The process of claim 5, wherein said organic solvent is selected from the

- 12 -

group of lower alcohols and acetone.

7. The process of claim 6, wherein said organic solvent is selected from the group of ethanol and ethyl acetate.

5

8. The process of any one of the claims 1-7, wherein the β -carotene extract is washed with water prior to crystallization.

9. The process of any one of the previous claims, wherein the natural 10 source is a microbial source.

10. The process of claim 9, wherein the microbial source is a fungus or an alga.

15 11. The process of claim 10, wherein the fungus is from the order *Mucorales*.

12. The process of claim 11, wherein the fungus is *Blakeslea trispora*.

20 13. A crystalline β -carotene preparation obtainable from a natural source with a purity of $\geq 95\%$, preferably $\geq 96\%$, more preferably $\geq 97\%$, even more preferably $\geq 98\%$, most preferably $\geq 99\%$.

14. The β -carotene preparation of claim 13, wherein the natural source is a 25 microbial source.

15. The β -carotene preparation of claim 14, wherein the microbial source is a fungus or an alga.

30 16. The β -carotene preparation of claim 15, wherein the fungus is from the order *Mucorales*.

- 13 -

17. The β -carotene preparation of claim 16, wherein the fungus is *Blakeslea trispora*.

18. A method for increasing the β -carotene content of β -carotene crystals by
5 treatment of said crystals with a solvent in which β -carotene has a low
solubility.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 97/03961

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C403/24 C12P23/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 064 645 A (BASF AG) 5 April 1967 see example 14 ---	1-18
X	US 3 268 606 A (JAEGER, H.J.) 23 August 1966 see the whole document ---	1-18
X	DATABASE WPI Week 9716 Derwent Publications Ltd., London, GB; AN 97-166034 XP002044040 "Extracting natural beta-carotene from carrot" & CN 1 092 063 A (SUN J) , 14 September 1994 see abstract -----	1-18



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

1

Date of the actual completion of the international search

Date of mailing of the international search report

20 October 1997

13. 11. 97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Janus, S

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. No

PCT/EP 97/03961

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 1064645 A		CH 433269 A FR 1403306 A NL 126583 C NL 6408209 A US 3356753 A	29-10-65 20-01-65 05-12-67

US 3268606 A	23-08-66	DE 1267644 B DK 111351 B FR 1409130 A GB 1019259 A NL 6411184 A	05-08-68 06-12-65 29-03-65
